

SEQUENCE LISTING

<110> Yacoby-Zeevi, Oron Peretz, Tuvia Miron, Daphna Shlomi, Yinon Pecker, Iris Ayal-Hershkovitz, Maty Feinstein, Elena Van Gelder, Joel M. Vlodavsky, Israel Friedmann, Yael <120> HEPARANASE ACTIVITY NEUTRALIZING ANTI-HEPARANASE MONOCLONAL ANTIBODY AND OTHER ANTI-HEPARANASE ANTIBODIES <130> 26128 <140> US 10/645,659 <141> 2003-08-22 <150> US 10/456,573 <151> 2003-06-09 <150> US 08/922,170 <151> 1997-11-02 <150> US 09/071,739 <151> 1998-05-01 <150> US 09/186,200 <151> 1998-11-04 <150> US 10/456,573 <151> 2003-06-09 <150> US 09/435,739 <151> 1999-03-01 <150> PCT/US98/17954 <151> 1998-08-31 <160> 16 <170> PatentIn version 3.3 <210> 1 <211> 386 <212> PRT <213> Homo sapiens <220> <221> misc_feature <223> $45~{\rm k\overline{D}a}$ subunit of mature processed heparanase dimer <400> 1

Lys Lys Phe Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu $1 \hspace{1.5cm} 5 \hspace{1.5cm} 10 \hspace{1.5cm} 15$

Tyr Thr Phe Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn 20 25 30

Ala Leu Leu Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln 35

Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu 50 60

Leu Gly Asn Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile

As Gly Ser Gln Leu Gly Glu Asp Phe Ile Gln Leu His Lys Leu Leu 85 90 95

65

Arg Lys Ser Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly 100 105 110

Gln Pro Arg Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala 115 120 125

Gly Gly Glu Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn 130 135 140

Gly Arg Thr Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp 145 150 155 160

Ile Phe Ile Ser Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr 165 170 175

Arg Pro Gly Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly
180 185 190

Gly Gly Ala Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp 195 200 205

Leu Asp Lys Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met 210 215 220

Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn 225 235 240

Phe Asp Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu 245 250 255

Val Gly Thr Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg 260 265 270

Lys Leu Arg Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr 275 280 285

Lys Glu Gly Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr 290 295 300

Lys Tyr Leu Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys 305 310 315 320

Tyr Leu Leu Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val

Gln Leu Asn Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro 340 345 350

Pro Leu Met Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro 355 360 365

Ala Phe Ser Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala 370 375 380

Cys Ile 385

<210> 2

<211> 535 <212> PRT

<213> Mus musculus

Met Leu Arg Leu Leu Leu Trp Leu Trp Gly Pro Leu Gly Ala Leu

Ala Gln Gly Ala Pro Ala Gly Thr Ala Pro Thr Asp Asp Val Val Asp

Leu Glu Phe Tyr Thr Lys Arg Pro Leu Arg Ser Val Ser Pro Ser Phe

Leu Ser Ile Thr Ile Asp Ala Ser Leu Ala Thr Asp Pro Arg Phe Leu

Thr Phe Leu Gly Ser Pro Arg Leu Arg Ala Leu Ala Arg Gly Leu Ser

Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr Asp Phe Leu Ile Phe

Asp Pro Asp Lys Glu Pro Thr Ser Glu Glu Arg Ser Tyr Trp Lys Ser

Gln Val Asn His Asp Ile Cys Arg Ser Glu Pro Val Ser Ala Ala Val

Leu Arg Lys Leu Gln Val Glu Trp Pro Phe Gln Glu Leu Leu Leu

Arg Glu Gln Tyr Gln Lys Glu Phe Lys Asn Ser Thr Tyr Ser Arg Ser

Ser Val Asp Met Leu Tyr Ser Phe Ala Lys Cys Ser Gly Leu Asp Leu

Ile Phe Gly Leu Asn Ala Leu Leu Arg Thr Pro Asp Leu Arg Trp Asn

Ser Ser Asn Ala Gln Leu Leu Leu Asp Tyr Cys Ser Ser Lys Gly Tyr

Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe Trp Lys Lys

Ala His Ile Leu Ile Asp Gly Leu Gln Leu Gly Glu Asp Phe Val Glu

Leu His Lys Leu Leu Gln Arg Ser Ala Phe Gln Asn Ala Lys Leu Tyr 250

Gly Pro Asp Ile Gly Gln Pro Arg Gly Lys Thr Val Lys Leu Leu Arg

Ser Phe Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Leu Thr Trp His

His Tyr Tyr Leu Asn Gly Arg Ile Ala Thr Lys Glu Asp Phe Leu Ser

Ser Asp Ala Leu Asp Thr Phe Ile Leu Ser Val Gln Lys Ile Leu Lys 310 315

Val Thr Lys Glu Ile Thr Pro Gly Lys Lys Val Trp Leu Gly Glu Thr

Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asn Thr Phe Ala

Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu Ser Ala Gln Met Gly

Ile Glu Val Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr His

Leu Val Asp Glu Asn Phe Glu Pro Leu Pro Asp Tyr Trp Leu Ser Leu 390 395

Leu Phe Lys Lys Leu Val Gly Pro Arg Val Leu Leu Ser Arg Val Lys 410

Gly Pro Asp Arg Ser Lys Leu Arg Val Tyr Leu His Cys Thr Asn Val

Tyr His Pro Arg Tyr Gln Glu Gly Asp Leu Thr Leu Tyr Val Leu Asn

Leu His Asn Val Thr Lys His Leu Lys Val Pro Pro Pro Leu Phe Arg 455

Lys Pro Val Asp Thr Tyr Leu Leu Lys Pro Ser Gly Pro Asp Gly Leu 470 475

Leu Ser Lys Ser Val Gln Leu Asn Gly Gln Ile Leu Lys Met Val Asp 490

Glu Gln Thr Leu Pro Ala Leu Thr Glu Lys Pro Leu Pro Ala Gly Ser 505

Ala Leu Ser Leu Pro Ala Phe Ser Tyr Gly Phe Phe Val Ile Arg Asn 520

Ala Lys Ile Ala Ala Cys Ile 530

<210> 3 <211> 536 <212> PRT

<213> Rattus norvegicus

Met Leu Arg Pro Leu Leu Leu Trp Leu Trp Gly Arg Leu Arg Ala 1 5 10 15

Leu Thr Gln Gly Thr Pro Ala Gly Thr Ala Pro Thr Lys Asp Val Val 20 25 30

Asp Leu Glu Phe Tyr Thr Lys Arg Leu Phe Gln Ser Val Ser Pro Ser 35 40 45

Phe Leu Ser Ile Thr Ile Asp Ala Ser Leu Ala Thr Asp Pro Arg Phe 50 55

Leu Thr Phe Leu Gly Ser Pro Arg Leu Arg Ala Leu Ala Arg Gly Leu 65 70 75 80

Ser Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr Asp Phe Leu Ile 85 90 95

Phe Asp Pro Asn Lys Glu Pro Thr Ser Glu Glu Arg Ser Tyr Trp Gln
100 105 110

Ser Gln Asp Asn Asn Asp Ile Cys Gly Ser Glu Arg Val Ser Ala Asp 115 120 125

Val Leu Arg Lys Leu Gln Met Glu Trp Pro Phe Gln Glu Leu Leu Leu 130 135 140

Leu Arg Glu Gln Tyr Gln Arg Glu Phe Lys Asn Ser Thr Tyr Ser Arg 145 150 155 160

Ser Ser Val Asp Met Leu Tyr Ser Phe Ala Lys Cys Ser Arg Leu Asp 165 170 175

Leu Ile Phe Gly Leu Asn Ala Leu Leu Arg Thr Pro Asp Leu Arg Trp 180 185 190

Asn Ser Ser Asn Ala Gln Leu Leu Leu Asn Tyr Cys Ser Ser Lys Gly 195 200 205

Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe Trp Lys. 210 215 220

Lys Ala Gln Ile Ser Ile Asp Gly Leu Gln Leu Gly Glu Asp Phe Val 225 230 235 240

Glu Leu His Lys Leu Leu Gln Lys Ser Ala Phe Gln Asn Ala Lys Leu 245 250 255

Tyr Gly Pro Asp Ile Gly Gln Pro Arg Gly Lys Thr Val Lys Leu Leu 260 265 270

Arg Ser Phe Leu Lys Ala Gly Gly Glu Val Ile Asp Ser Leu Thr Trp 275 280 285

His His Tyr Tyr Leu Asn Gly Arg Val Ala Thr Lys Glu Asp Phe Leu 290 295 300 Ser Ser Asp Val Leu Asp Thr Phe Ile Leu Ser Val Gln Lys Ile Leu 310

Lys Val Thr Lys Glu Met Thr Pro Gly Lys Lys Val Trp Leu Gly Glu

Thr Ser Ser Ala Tyr Gly Gly Gly Ala Pro Leu Leu Ser Asn Thr Phe

Ala Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu Ser Ala Gln Leu

Gly Ile Glu Val Val Met Arg Gln Val Phe Phe Gly Ala Gly Asn Tyr

His Leu Val Asp Glu Asn Phe Glu Pro Leu Pro Asp Tyr Trp Leu Ser

Leu Leu Phe Lys Lys Leu Val Gly Pro Lys Val Leu Met Ser Arg Val 410

Lys Gly Pro Asp Arg Ser Lys Leu Arg Val Tyr Leu His Cys Thr Asn 425

Val Tyr His Pro Arg Tyr Arg Glu Gly Asp Leu Thr Leu Tyr Val Leu 440

Asn Leu His Asn Val Thr Lys His Leu Lys Leu Pro Pro Pro Met Phe

Ser Arg Pro Val Asp Lys Tyr Leu Leu Lys Pro Phe Gly Ser Asp Gly 470

Leu Leu Ser Lys Ser Val Gln Leu Asn Gly Gln Thr Leu Lys Met Val

Asp Glu Gln Thr Leu Pro Ala Leu Thr Glu Lys Pro Leu Pro Ala Gly 500 505

Ser Ser Leu Ser Val Pro Ala Phe Ser Tyr Gly Phe Phe Val Ile Arg

Asn Ala Lys Ile Ala Ala Cys Ile

<210> 4

<211> 543 <212> PRT <213> Homo sapiens

<400> 4

Met Leu Leu Arg Ser Lys Pro Ala Leu Pro Pro Pro Leu Met Leu Leu 10

Leu Leu Gly Pro Leu Gly Pro Leu Ser Pro Gly Ala Leu Pro Arg Pro

Ala Gln Ala Gln Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro

Leu His Leu Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp Ala Asn 50 60

Leu Ala Thr Asp Pro Arg Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu 65 70 75 80

Arg Thr Leu Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly
85 90 95

Thr Lys Thr Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu Ser Thr Phe 100 105 110

Glu Glu Arg Ser Tyr Trp Gln Ser Gln Val Asn Gln Asp Ile Cys Lys 115 120 125

Tyr Gly Ser Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp 130 135 140

Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys Lys Phe 145 150 155 160

Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe 165 170 175

Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu Leu 180 185 190

Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu . 195 200 205

Asp Tyr Cys Ser Ser Lys Gly Tyr Asn.Ile Ser Trp Glu Leu Gly Asn 210 215 220

Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser 225 230 235 240

Gln Leu Gly Glu Asp Phe Ile Gln Leu His Lys Leu Leu Arg Lys Ser

Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg 260 265 270

Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Glu 275 280 285

Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr 290 295 300

Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile 305 310 315 320

Ser Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly 325 330 335

Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly Gly Ala 340 345 350

Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys 360

Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val 375

Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro

Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr 410

Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg 425

Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly 440

Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu 455

Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu 470

Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn

Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met 505

Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser

Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala Cys Ile 535

<210> 5

<211> 523 <212> PRT

<213> Gallus gallus

<400> 5

Met Leu Val Leu Leu Leu Val Leu Leu Leu Ala Val Pro Pro Arg

Arg Thr Ala Glu Leu Gln Leu Gly Leu Arg Glu Pro Ile Gly Ala Val

Ser Pro Ala Phe Leu Ser Leu Thr Leu Asp Ala Ser Leu Ala Arg Asp 40

Pro Arg Phe Val Ala Leu Leu Arg His Pro Lys Leu His Thr Leu Ala

Ser Gly Leu Ser Pro Gly Phe Leu Arg Phe Gly Gly Thr Ser Thr Asp

Phe Leu Ile Phe Asn Pro Asn Lys Asp Ser Thr Trp Glu Glu Lys Val 85 90 95

Leu Ser Glu Phe Gln Ala Lys Asp Val Cys Glu Ala Trp Pro Ser Phe 100 105 110

Ala Val Pro Lys Leu Leu Thr Gln Trp Pro Leu Gln Glu Lys 115 120 125

Leu Leu Leu Ala Glu His Ser Trp Lys Lys His Lys Asn Thr Thr Ile 130 135 140

Thr Arg Ser Thr Leu Asp Ile Leu His Thr Phe Ala Ser Ser Ser Gly 145 150 155 160

Phe Arg Leu Val Phe Gly Leu Asn Ala Leu Leu Arg Arg Ala Gly Leu 165 170 175

Gln Trp Asp Ser Ser Asn Ala Lys Gln Leu Leu Gly Tyr Cys Ala Gln 180 185 190

Arg Ser Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn Ser Phe 195 200 205

Arg Lys Lys Ser Gly Ile Cys Ile Asp Gly Phe Gln Leu Gly Arg Asp 210 215 220

Phe Val His Leu Arg Gln Leu Leu Ser Gln His Pro Leu Tyr Arg His 225 230 235 240

Ala Glu Leu Tyr Gly Leu Asp Val Gly Gln Pro Arg Lys His Thr Gln
245 250 255

His Leu Leu Arg Ser Phe Met Lys Ser Gly Gly Lys Ala Ile Asp Ser 260 265 270

Val Thr Trp His His Tyr Tyr Val Asn Gly Arg Ser Ala Thr Arg Glu 275 280 285

Asp Phe Leu Ser Pro Glu Val Leu Asp Ser Phe Ala Thr Ala Ile His 290 295 300

Asp Val Leu Gly Ile Val Glu Ala Thr Val Pro Gly Lys Lys Val Trp 305 310 315 320

Leu Gly Glu Thr Gly Ser Ala Tyr Gly Gly Gly Ala Pro Gln Leu Ser 325 330 335

Asn Thr Tyr Val Ala Gly Phe Met Trp Leu Asp Lys Leu Gly Leu Ala 340 345 350

Ala Arg Arg Gly Ile Asp Val Val Met Arg Gln Val Ser Phe Gly Ala 355 360 365

Gly Ser Tyr His Leu Val Asp Ala Gly Phe Lys Pro Leu Pro Asp Tyr 370 375 380

Trp Leu Ser Leu Leu Tyr Lys Arg Leu Val Gly Thr Arg Val Leu Gln

Ala Ser Val Glu Gln Ala Asp Ala Arg Arg Pro Arg Val Tyr Leu His

Cys Thr Asn Pro Arg His Pro Lys Tyr Arg Glu Gly Asp Val Thr Leu

Phe Ala Leu Asn Leu Ser Asn Val Thr Gln Ser Leu Gln Leu Pro Lys

Gln Leu Trp Ser Lys Ser Val Asp Gln Tyr Leu Leu Pro His Gly

Lys Asp Ser Ile Leu Ser Arg Glu Val Gln Leu Asn Gly Arg Leu Leu

Gln Met Val Asp Asp Glu Thr Leu Pro Ala Leu His Glu Met Ala Leu

Ala Pro Gly Ser Thr Leu Gly Leu Pro Ala Phe Ser Tyr Gly Phe Tyr

Val Ile Arg Asn Ala Lys Ala Ile Ala Cys Ile

<210> 6

<211> 10 <212> PRT

<213> Artificial sequence

<223> Functional peptide epitope of heparanase

<400> 6

Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys

<210> 7

<211> 19 <212> PRT

<213> Artificial sequence

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<223> Functional peptide epitope of heparanase

<400> 7

Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr Asp Phe Leu Ile Phe

Asp Pro Lys

<210> 8

<211> 15 <212> PRT

<213> Artificial sequence

<223> Functional peptide epitope of heparanase

<400> 8

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<211> 15
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<223> Functional peptide epitope of heparanase
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Arg Pro Gly Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr
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<211> 14
<212> PRT
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<223> Functional peptide epitope of heparanase
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Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr Ala Thr Arg
<210> 11
<211> 74
<212> PRT
<213> Homo sapiens
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<221> misc feature
<223> 8~{\rm kDa} subunit of mature processed heparanase dimer
Gln Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro Leu His Leu
                                     10
Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp Ala Asn Leu Ala Thr
Asp Pro Arg Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu Arg Thr Leu
        35
                             40
Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly Thr Lys Thr
                        55
Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu
                     70
<210> 12
<211> 8
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<223> HS-binding protein consensus sequence
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<222> (1)..(1)
<223> Xaa can be any naturally occurring amino acid
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<220>
<221> misc_feature
<222> (2)..(4)
<223> Basic amino acid residue
<220>
<221> misc_feature
<222>
      (5)..(6)
<223> Xaa can be any naturally occurring amino acid
<220>
<221> misc_feature
<222> (7)..(7)
<223> Basic amino acid residue
<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa can be any naturally occurring amino acid
<400> 12
Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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<211> 6
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<223> HS-binding protein consensus sequence
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<223> Xaa can be any naturally occurring amino acid
<220>
<221> misc_feature
<222> (2)..(3)
<223> Basic amino acid residue
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<221> misc_feature
<222> (4)..(4)
<223> Xaa can be any naturally occurring amino acid
<220>
<221> misc_feature
<222> (5)..(5)
<223> Basic amino acid residue
<220>
<221> misc_feature
<222>
       (6)..(6)
<223> Xaa can be any naturally occurring amino acid
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Xaa Xaa Xaa Xaa Xaa
<210> 14
<211> 6
<212> PRT
<213> Homo sapiens
<400> 14
Gln Lys Lys Phe Lys Asn
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